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I verify that the attached English translation is a true and correct translation made by me of a certified copy in the German language of European patent application No 99120351.4;

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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M W R Turner

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Certificate

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Patent application No

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Improved method of detecting acid-resistant microorganisms in the stool

The description of this invention mentions a number of published documents. The subject-matter of those documents is incorporated into the description by reference.

The invention relates to a method of detecting an infection of a mammal with an acid-resistant microorganism, wherein (a) a stool sample of the mammal is incubated with (aa) a receptor under conditions permitting a complex formation of an antigen from the acid-resistant microorganism with the receptor; or (ab) two different receptors under conditions permitting a complex formation of an antigen from the acid-resistant microorganism with the two receptors and wherein the receptor according to (aa) or the receptors according to (ab) specifically bind(s) an antigen which, at least with some mammals, has a structure after passage through the intestine that corresponds to the native structure or the structure against which a mammal produces antibodies after being infected or immunized with the acid-resistant microorganism or an extract or lysate thereof or a protein therefrom or a fragment thereof or a synthetic peptide; and (b) the formation of at least one antigen-receptor complex according to (a) is detected. Preferably, the acid-resistant microorganism is a bacterium, in particular *Helicobacter pylori, Helicobacter hepaticus, Campylobacter jejuni* or *Mycobacterium tuberculosis*. Moreover, the receptor(s) preferably bind(s) to (an) epitope(s) of a catalase. Furthermore, the invention relates to diagnostic and pharmaceutical compositions and test devices containing said components and packages containing the same.

Today, there are various invasive, semi-invasive or non-invasive methods of detecting the infection of a mammal organism with a microbial pathogen or parasite. All invasive methods presuppose endoscopy and biopsy. If these techniques are used, the physical integrity of the

examined subject is violated, e.g. in a biopsy. Obtaining a specimen by biopsy is time-consuming, costly and mostly involves a high strain on the patient. As the infection with particular microorganisms, for instance with *H. pylori*, need not be distributed over the entire gastric mucosa, obtaining a specimen by biopsy at a non-infected site may deliver a false-negative result. Another disadvantage of all invasive methods is that the examination results are influenced by earlier treatment with proton-pump inhibitors, bismuth or antibodies.

Semi-invasive or non-invasive diagnostic methods note changes in parameters which may be measured without interfering in the organism. For this purpose preferably samples of body fluids and excretions, such as serum, breath, urine, saliva, sweat or stool are taken and analysed. With direct methods the presence of the pathogen or parasite, its components or their degradation products is detected by electron microscopy, optical characterisation, mass spectrometry, measurement of the radioactive degradation products or specific enzymatic reactions. However, these methods often require expensive and sophisticated instruments (e.g. the breath test). By contrast, indirect methods are used for detecting reactions of the host organism to the pathogen or the parasite, for instance the presence of antibodies against antigens of the pathogen in the serum or the saliva of the host. Since interfering in the organism using invasive techniques strains the organism in most cases and frequently also requires expensive and sophisticated instruments and involves a health hazard, non-invasive techniques are the methods of choice since it is comparatively simple to take samples of the above-mentioned body fluids and excretions. Furthermore, since not every host reacts in the same way to a given pathogen or parasite, and the host reaction is delayed and also may persist even after the pathogen or parasite has been removed from the organism, direct methods are always to be preferred. Hence, ideally, a diagnosis is made by means of the non-invasive, direct detection of the pathogen or parasite in body fluids or excretions. Contrary to indirect methods, this allows the current infection status to be determined.

Moreover, a diagnostic method should also be optimised with regard to other aspects: high reproducibility, sensitivity and specificity, guaranteed availability and constant quality of the materials to be used, low costs in production and carrying out the method and simple application independent of expensive and sophisticated instruments are the parameters to be taken into consideration here.

For the above-mentioned reasons, in medical diagnostics increasing use is being made of methods based on the high selectivity and binding affinity of given classes of substances (e.g. antibodies, receptors, lectins and aptamers) for molecular structures which can be selected in such a way that they are highly specific for the corresponding substance to be analysed. It was in particular the possibility of immobilising those substances on surfaces of solids as well as the coupling of radioactive nuclides, of enzymes triggering colour reactions with suitable substrates, or coloured particles with a highly specific binding affinity (e.g. ELISA = enzyme-linked immunosorbent assay) that led to the development of inexpensive, simple and less time-consuming methods of detecting substances that are naturally occurring in the body or foreign to the body.

In the initial phases of the development of those detection methods exclusively polyclonal antibodies were used. They have some disadvantages well known to the man skilled in the art however, thus mainly limited availability and often also cross-reactivity. The development of methods of preparing monoclonal antibodies (Köhler & Milstein (1975)), the advances in the isolation of receptors and their directed expression in cellular host systems, the development of lectins with high affinity to given carbohydrates and the discovery that single-stranded nucleic acid molecules (aptamers) are able to specifically bind molecular structures, allowed the majority of those disadvantages to be eliminated. Today, the specificity and sensitivity of detection methods can be optimised with comparatively simple methods.

Due to the high specificity, such methods are particularly suitable for detecting individual defined substances such as haptens, peptides or

proteins, provided the structural element that has been recognised is constant within the specimen population to be examined and specific to the substance to be detected. Moreover they are well suited for measurements in body fluids and thus are an obvious option for the direct detection of pathogens in that specimen matrix. Accordingly, the prior art describes methods of diagnosing e.g. *Entamoeba histolytica* (Hague (1993), J. Infect. Dis. 167: 247-9), enterohemorrhagic *Escherichia coli* (EHEC, Park (1996), J. Clin. Microbiol. 34: 988-990), *Vibrio cholerae* (Hasan (1954), FEMS Microbiol. Lett. 120: 143-148), Toro virus-like particles (Koopmans (1993) J. Clin Microbiol. 31: 2738-2744) or *Taenia saginata* (Machnicka (1996), Appl. Parasitol. 37: 106-110) from stool.

The feature that the above-described pathogens have in common is that they are viable and reproducible in the intestine of the host, in all cases humans. Hence they have mechanisms allowing them to survive and propagate in the presence of the degradation and digestion systems active in the intestine. Thus it is probable that a large number of intact or almost intact pathogens or parasites are passed upon excretion with the stool. As a rule, it is easy to detect them in the stool or in prepared stool samples by means of detection reagents, for instance antibodies that recognise the intact pathogens or parasites.

There is however a number of pathogens or parasites that on the one hand may be present in the stool due to the relations of the affected tissue (e.g. lungs, stomach, pancreas, duodenum, liver) to the gastrointestinal tract and that on the other hand are however not viable and/or reproducible in the intestine itself. These pathogens and parasites include, for instance, *Helicobacter pylori (H. pylori)* and *Helicobacter hepatis, Mycobacterium tuberculosis* and other mycobacteria, *Chlamydia pneumoniae, Legionella pneumophilae, Pneumocystis carinii* and others. Some of these pathogens can be detected for example in the sputum, however for example the detection of *Mycobacterium tuberculosis* in the sputum is possible only during a short period of time, more specifically once a cavity containing the pathogen has opened. Moreover detection is

rendered more difficult by the fact that it is not always possible to obtain a sputum sample from the subject to be examined. This applies for instance to infants, confused patients or animals. Other pathogens such as *Legionella pneumophilae* can be detected specifically by means of antigens which pass into the urine via the kidneys. That however is only possible if the amount in the urine is sufficient for detection. Detection in the stool would be a welcome alternative to that. In these organisms however passage through the intestine is combined with a strong attack by the digestion and degradation mechanisms of the intestinal flora. In this case, molecular structures which are specific to the pathogen observed can be destroyed or their concentration can be greatly reduced.

With other acid-resistant bacteria too degradation of the pathogens in the intestine has turned out to be a problem for reliable detection in stool samples. The number of germs in the stomach of an infected patient is small compared to other bacteria settling in the intestine. Furthermore, germs and germ fragments have to pass a long way through the intestine which is rich in proteases after leaving the stomach. Those circumstances mean that only small amounts of intact proteins can be found in the stool, in which respect it cannot however be assumed that it is always the same fragments of specific proteins that pass through the intestinal tract undamaged. Another consequence of this is that the combination of two epitopes on one antigen, which is necessary for an ELISA test, is no longer necessarily like the one occurring in the native protein and epitopes located close to each other are most likely to show a positive result in a detection method requiring two epitopes on the same molecule. Ideally, only one epitope on the same molecule is needed for detection. In addition, the individually different distribution of antigens detected in the stool of infected patients suggests individual features in processing of the antigens during passage through the intestine. A first approach to reducing this problem was provided by the disclosure of EP-A 0 806 667. In that application it was shown that polyclonal antibodies could be induced with the lysate of a given *H. pylori* strain, which recognise a greater variability of

strains from different geographical regions. However this application does not indicate which antigens are recognised by the serum. In view of the fact that immune sera may vary in spite of all standardisation efforts, the method developed in the above-mentioned application must be regarded as suboptimal for broad application. In addition, it is necessary to repeatedly immunise new animals in order to provide the polyclonal sera. The corresponding methods are both time-consuming and costly.

Ideally reliable detection of the infection of an acid-resistant pathogenic organism/parasite as broadened above is possible with a single or a limited number of reagent(s) specific to that pathogenic organism/parasite. Such a possibility would above all considerably reduce the costs of corresponding detection methods. Hence, the underlying problem of the present invention was to provide a corresponding detection method or corresponding reagents.

That problem is solved by the embodiments characterised in the claims.

Thus, the invention relates to a method of detecting an infection of a mammal with an acid-resistant microorganism, wherein (a) a stool sample of the mammal is incubated with (aa) a receptor under conditions permitting a complex formation of an antigen from the acid-resistant microorganism with the receptor; or (ab) two different receptors under conditions permitting a complex formation of an antigen from the acid-resistant microorganism with the two receptors, and wherein the receptor according to (aa) or the receptors according to (ab) specifically bind(s) an antigen which at least in some of the mammals has a structure after passage through the intestine that corresponds to the native structure or the structure against which a mammal produces antibodies after being infected or immunised with the acid-resistant microorganism or an extract or lysate thereof or a protein therefrom or a fragment thereof or a synthetic peptide; and (b) the formation of at least one antigen-receptor complex according to (a) is detected.

In accordance with the present invention, the term "acid-resistant microorganism" encompasses any microorganism which, due to its properties/mechanisms of adapting to the host, withstands the physical and chemical influences of the digestive tract so that it can be detected by a preferably immunological test or by the use of aptamers. Examples of such acid-resistant microorganisms are *Helicobacter pylori, Helicobacter hepaticum, Mycobacterium tuberculosis, Mycobacterium pseudotuberculosis* and *Mycobacterium cansassii*.

The term "stool sample of the mammal" in accordance with the present invention means any stool sample which can be used for the detection method of the invention. In particular it includes stool samples which have been prepared for diagnostic tests according to per se known methods. Preparation is carried out for instance according to RIDASCREEN® Entamoeba enzyme immunoassay (R-Biopharm GmbH, Darmstadt).

The man skilled in the art can readily adjust "conditions permitting complex formation", cf. also Harlow and Lane, ibid. These conditions are for example physiological conditions.

The term "has a structure after passage through the intestine that corresponds to the native structure", in accordance with the present invention, means that the epitope of an antigen is recognised after passage through the intestine by a receptor, e.g. a monoclonal antibody, derivative or fragment thereof or the aptamer which was obtained against the same antigen/epitope that has not passed the intestine or which binds thereto. In other words, the epitope/antigen that is specifically bound by the above has passed the intestine intact or essentially intact as regards its structure and has not been degraded. A source for the native structure of the epitope/antigen may be for instance a bacterial extract that was disrupted by means of a French press and further purified with standard methods (cf., for instance, Sambrook et al., "Molecular Cloning, A Laboratory Manual", 2nd edition, 1989, CSH Press, Cold Spring Harbor USA) or a

bacterial lysate further purified according to standard methods (e.g. Sambrook et al., ibid.).

According to the invention, the term "has a structure after passage through the intestine that corresponds to the structure against which a mammal produces antibodies after being infected or immunised with the acid-resistant microorganism or an extract or lysate thereof or a protein therefrom or a fragment thereof or a synthetic peptide" means that the epitope recognised by the receptor corresponds to an epitope which is presented by the immune system of a mammal, preferably a human. The mechanisms of antigen presentation as well as the mechanisms leading to the processing of antigens and the variety of antibodies resulting therefrom are known in the prior art and are described for instance in Janeway and Travers, Immunologie, 2nd edition 1997, Spektrum Akademischer Verlage GmbH, Heidelberg. Those epitopes may differ from native epitopes. Contact of the mammal with the microorganisms or proteins or fragments or the synthetic peptides can be brought about by natural infection (except for synthetic peptides) or by immunisation. For immunisation extracts, lysates, synthetic peptides, etc. of the microorganism/protein can also be used. Suitable immunisation methods are known in the prior art and are described for instance in Harlow and Lane, ibid. Suitable antibodies may also be obtained for example by immunisation and/or screening for surrogates such as synthetic peptides, recombinantly produced proteins, extracts, lysates or partially digested proteins.

"Synthetic peptides" comprise peptides having at least one epitope of the native antigen or the antigen which has passed through the intestine. The peptides can have the same primary structure as the antigen or fragments thereof. However they can also have a different primary structure (primary amino acid sequence, for instance conservative exchanges).

The terms "specifically binds" means according to the invention that the receptor shows no or essentially no reactivity with other epitopes in samples of non-infected mammals.

In this embodiment of the invention a prepared stool sample can be bound for instance to a solid phase and the infecting agent can be detected with the labelled receptor. If the antigen which is present after having passed the intestine is (still) present in (homo-)dimeric or multimeric form, the same receptor can be used both as a catcher and as a detector.

In addition, it is of importance for the method of the invention that successful detection requires only one epitope of an antigen protein to be detectable after passage through the intestine in an essentially consistent manner. That epitope can also occur several times on a homo-dimer or -multimer. The likelihood of finding that epitope in detectable form is however significantly higher than if a detection test has to rely on more than one epitope to be detected.

Finally the method of the invention requiring only one receptor involves advantages as regards costs and standardisation.

On the basis of the surprising finding according to the invention that given antigens from said microorganisms have an epitope structure after passage through the intestine which can be essentially consistently detected a second embodiment must also be considered essential to the invention. This embodiment is based on the fact that different receptors bind to different epitopes of the same antigen. Here the term "essentially" means that the epitope(s) and thus a corresponding infection with the microorganism can be detected in more than 70%, preferably at least 75%, more preferably more than 85%, particularly preferred more than 90%, even more preferably more than 95% and most preferably more than 98% of the infected individuals. Ideally infections are detected in 100% of the infected individuals.

According to the invention it was surprisingly found that by means of a single receptor which specifically binds an epitope of an antigen of an acid-resistant microorganism, or two receptors which specifically bind two epitopes of the same antigen infection with those bacteria/pathogens can be relatively reliably diagnosed. The invention includes embodiments in which other epitopes having said properties are recognised by other

receptors, for instance by monoclonal antibodies or fragments or derivatives thereof or aptamers. The latter embodiments are suitable for further increasing the reliability of the diagnosis. Advantageously those other receptors may be antibodies, fragments or derivatives, which specifically recognise urease, preferably ß-urease, the 26 kDa protein or Hsp 60, all preferably from *H. pylori*. The detection of one or more of those proteins/protein fragments may be carried out in the same test or in an independent test with another part of the same sample.

The results of the invention are surprising mainly because the state of the art had taught away therefrom. In the case of *H. pylori* for example it was found that main antigens do not show the desired specificity and sensitivity in ELISA tests; cf. Newell et al., Serodiag. Immunother. Infect. Dis. 3 (1989), 1-6. Moreover, EP-A-0 806 667 teaches that it is not possible to reliably detect *H. pylori* infections with receptors such as monoclonal antibodies due to the genetic variability of the *H. pylori* strains.

Compared to the aforementioned state of the art the method of the invention is of advantage in particular since it permits a relatively reliable diagnosis with only one receptor. In ELISA for instance pairs of receptors such as antibodies, fragments, derivatives thereof or aptamers are preferably used for detection, with the two receptors of the pair binding the same or different epitopes on the same antigen. *H. pylori* catalase for example forms multimeric structures of several identical subunits. Therefore in ELISA or other assays the same receptors can be used both as catching receptors and also as detection receptors. Another advantage of the method of the invention is the fact that it is a direct and non-invasive method, which increases the above-mentioned advantages for the patient and reliability in determining the stage of the disease.

In a preferred embodiment the acid-resistant microorganism is an acid-resistant bacterium.

A number of acid-resistant bacteria are known in the state of the art. In a particularly preferred embodiment the acid-resistant bacterium is a

bacterium of the genus Helicobacter, Campylobacter or the genus Mycobacterium.

In another particularly preferred embodiment the bacterium is a bacterium of the species *Helicobacter pylori*, *Helicobacter hepaticum*, *Campylobacter jejuni* or a bacterium of the species *Mycobacterium tuberculosis*.

In another particularly preferred embodiment the receptor(s) is/are (an) antibody (antibodies), (a) fragment(s) or (a) derivative(s) thereof or (an) aptamer(s).

In accordance with the present invention, "fragments" or "derivatives" of monoclonal antibodies have the same binding specificity as the monoclonal antibodies. Such fragments or derivatives can be produced according to usual methods; cf. for example Harlow and Lane, "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, USA, 1988. Examples of fragments include Fab-, $F(ab')_2$ or Fv-fragments. ScFv-fragments are examples of derivatives. Derivatives can also be chemically produced substances having the same binding properties as the antibodies or improved binding properties. Such substances can be produced for instance by peptidomimetics or by different cycles of phage display and subsequent selection to improved binding properties. According to the invention, aptamers are nucleic acids such as RNA; ssDNA (ss=single stranded), modified RNA or modified ssDNA, which bind a large number of target sequences having high specificity and affinity. The term "aptamer" is known and defined in the state of the art, for example in Osborne et al., Curr. Opin. Chem. Biol. 1 (1997), 5-9 or in Stull and Szoka, Pharm. Res. 12 (1995), 465-483.

The term "antigen-antibody complex" in accordance with the present invention includes not only complexes which the antigen forms with the native antibody, but also those which it forms with the fragments or derivatives thereof.

The invention includes embodiments in which only monoclonal antibodies or fragments or derivatives thereof or only aptamers are used as

well as embodiments in which different types of detection reagents are used in one test. Hence it is possible for a first monoclonal antibody to be used with a second antibody derivative or a first aptamer to be used with a second antibody fragment, to name only two examples. In this respect, the terms "first" and "second" refer to the first and the second detection reagent. That however does not mean that two antibodies, derivatives or fragments thereof or two aptamers are always used.

The use of monoclonal antibodies, fragments or derivatives thereof or of aptamers ensures a standard which is to be easily observed in reliability of the diagnosis method, which means a great advantage compared to diagnosis methods that have been known hitherto and that have been introduced for this purpose. Moreover it is no longer necessary to keep re-immunising and subsequently testing test animals as is required for instance in the method according to EP-A 0 806 667.

In another preferred embodiment the antigen is the antigen of a catalase, preferably from *H. pylori.* The catalase has the special advantage that it could be detected in all acid-resistant bacteria known hitherto. According to the invention it was found, as another advantage, that the catalase is highly resistant to digestion in the intestinal tract, which simplifies detection of significant amounts. Finally the catalase or fragments thereof is/are often still present in a superior structure, for instance in tetrameric form, after having passed through the intestine, which facilitates detection with one receptor type only.

According to the invention, it was surprisingly found that in a population of mammals, in particular human patients, whose stool had been tested for infections with acid-resistant bacteria, essentially all members of this population showed consistently recurring catalase epitopes in the stool, so that a relatively reliable diagnosis can be made with a high degree of probability with only one corresponding receptor, preferably monoclonal antibodies, fragments or derivatives thereof or aptamers. In particular, since the catalase has a tetrameric antigen structure, this

diagnosis can advantageously be made for instance in ELISA or in similarly arranged solid systems.

It is particularly preferred for the catalase to be the catalase of *H. pylori*.

In another preferred embodiment additional use is made of a mixture of receptors for detection, with the mixture of receptors having the function of a catcher of the antigen if the receptor is used as detector of the antigen, and the mixture having the function of a detector of the antigen if the receptor is used as catcher of the antigen. This embodiment of the invention permits particularly reliable diagnosis, particularly if the antigen is not present in a dimeric or multimeric conformation after passing through the intestine. This embodiment makes it possible for only one of the two receptor types used in the majority of standardized immunological detection methods to be a monoclonal antibody while for instance the second receptor type may be a polyclonal serum.

In a particularly preferred embodiment the mixture of receptors is a polyclonal antiserum.

In an additionally particularly preferred embodiment the polyclonal antiserum against a lysate of the microorganism, preferably *H. pylori*, was obtained.

In another particularly preferred embodiment the lysate is a lysate with an enriched antigen.

In another preferred embodiment, the lysate is a lysate with depleted immunodominant antigen.

The two aforementioned embodiments also include the fact that the lysate is a lysate with enriched antigen, preferably with enriched catalase and with depleted immunodominant antigen, preferably mainly antigenic urease. In particular that combination offers the possibility of obtaining a high immunization yield which is especially suitable for the method of the invention. A way of carrying out corresponding enrichment and depletion methods is described in greater detail in the Examples.

The polyclonal antiserum against a purified or a (semi)synthetically produced antigen was obtained according to another particularly preferred embodiment.

According to the invention, the receptors, preferably the monoclonal antibodies, fragments or derivatives thereof or the aptamers, can recognise and specifically bind linear or conformation epitopes. In another preferred embodiment, at least one of the receptors binds a conformation epitope.

In a particularly preferred embodiment, all receptors bind conformation epitopes.

In a particularly preferred embodiment, the heavy chain of the antibody binding a catalase epitope has at least one of the following CDRs, preferably the CDR3 and still more preferably all of the following three CDRs:

CDR1: NYWIH

CDR2: YINPATGSTSYNQDFQD

CDR3: EGYDGFDS

In another particularly preferred embodiment, the DNA sequence encoding the heavy chain of the antibody binding a catalase epitope has at least one of the following CDRs, preferably the CDR3 and still more preferably all of the following three CDRs:

CDR1: AACTACTGGA TTCAC

CDR2: TACATTAATC CTGCCACTGG TTCCACTTCT TACAATCAGG ACTTTCAGGA C

CDR3: GAGGGGTACG ACGGGTTTGA CTCC

In another particularly preferred embodiment, the light chain of the antibody binding a catalase epitope has at least one of the following CDRs, preferably the CDR3 and still more preferably all of the following three CDRs:

CDR1: SASSSVNYMY

CDR2: DTSKLAS

CDR3: QQWSSNPYT

Furthermore, in another particularly preferred embodiment, the DNA sequence encoding the light chain of this antibody has at least one of the following CDRs, preferably the CDR3 and still more preferably all of the following three CDRs:

CDR1: AGTGCCAGCT CAAGTGTAAA TTACATGTAC

CDR2: GACACATCCA AATTGGCTTC T

CDR3: CAGCAGTGGA GTAGTAATCC GTACACG

In addition, it is particularly preferred that the heavy and light chains having said CDRs occur together in one antibody, fragment or derivative thereof, which specifically binds the catalase or a fragment thereof, preferably from *H. pylori*. However the invention also comprises embodiments in which those heavy or light chains are combined with other light or heavy chains, wherein the binding properties may essentially be maintained or improved. Corresponding methods are known in the prior art. Particularly preferred antibodies have in the variable regions of the light and heavy chains the amino acid sequences shown in Figures 1 and 2 or the regions are encoded by the DNA sequences shown therein.

In a preferred embodiment, the following steps are carried out with the stool sample before incubation with the antibodies: the stool sample is resuspended in a resuspension buffer at a ratio of 1:3 to 1:25, preferably about 1:10, and then mixed on a vortex mixer. An example of a resuspension buffer is 150 mM PBS, 0.1% SDS.

In another preferred embodiment, the formation of the at least one antigen-receptor complex/antigen-receptor-receptor-mixture complex in step (b) is detected by means of an immunologic method.

In another preferred embodiment, the formation of the at least one antigen-receiver complex/antigen-receptor-receptor-mixture complex in step (b) is detected by means of ELISA, RIA, Western Blot or an immunochromatographic method.

Such methods are known per se in the state of the art; cf. Harlow and Lane, ibid.

In a particularly preferred embodiment of the method of the invention, in the immunological method, in particular in RIA or ELISA, the same receptor is used for both binding to the solid phase and for detecting the epitope. While the catching receptor can be bound to the solid phase, e.g. a microtitre plate, in unmodified form, the receptor used for detection may optionally be labelled. On the other hand that receptor may also not be labelled and thus the epitope of the microorganism, preferably the bacterial epitope, may be detected via a third labelled receptor, that receptor preferably being an antibody, fragment or derivative thereof or an aptamer, or a species-specific or Ig class-specific antibody or a corresponding aptamer. Labellings of antibodies, for example with radioactive or fluorescent markers are known in the state of the art; cf. Harlow and Lane, ibid. The same applies to aptamers. The above-described embodiment is particularly suitable for detecting the catalase which may optionally also be present as a tetramer after passage through the intestine. It will be appreciated that in this embodiment combinations of antibodies, fragments, derivatives and aptamers can also be used, e.g. combinations of antibodies etc. which bind to different epitopes of the same antigen.

In another preferred embodiment of the method of the invention the monoclonal antibody is a murine antibody.

In addition, in another preferred embodiment the receptors are fixed to a support.

When carrying out routine checks, it is of particular advantage to fix the receptors, preferably the antibodies, fragments or derivatives thereof or the aptamers to a support. Moreover the combination antibody-support/aptamer-support may be packaged as a tool set or in the form of a kit.

In a particularly preferred embodiment the material of the support is a porous support material.

In another particularly preferred embodiment the support material is a test strip.

In addition in a preferred embodiment the support material consists of cellulose or a cellulose derivative.

The mammal whose stool can be analysed by means of the method of the invention may be an animal, e.g. a domestic animal such as a cat or a dog, a useful animal such as a pig or another kind of animal such as a mouse, a tiger or a ferret.

In a preferred embodiment, the mammal is a human.

Furthermore, the invention relates to a monoclonal antibody, a fragment or derivative thereof having a V-region which has a combination of the aforementioned CDRs or which is produced by one of the aforementioned hybridomas.

In that respect a preferred monoclonal antibody, fragment or derivative is one which has at least one of the V-regions shown in Figures 1 and 2. Preferably, that antibody has two of the V-regions shown in Figures 1 and 2. Moreover those V-regions are preferred to be encoded by the DNA sequences shown in Figures 1 and 2.

In a particularly preferred embodiment of the invention the monoclonal antibody, the fragment or derivative thereof is a murine antibody or a fragment or derivative thereof or a chimeric, preferably a humanized antibody or a fragment or derivative thereof. The derivative may also be a fusion protein. Furthermore the antibody is preferably labelled, for instance with a colloid, with a radioactive, fluorescent, phosphorescent or chemiluminescent labelling.

The production of chimeric humanized and human antibodies and of the other derivatives is well known in the state of the art (e.g. Vaughan et al., 1998; Orlandi et al., 1989, Harlow and Lane, ibid.).

The invention also relates to an aptamer which specifically binds the same epitope as the monoclonal antibody, the fragment or derivative thereof. Such aptamers can be produced with methods known in the state of the art.

In addition, the invention relates to an epitope which is specifically bound by one of the above-described monoclonal antibodies, fragments or derivatives thereof or aptamers.

Furthermore, the invention relates to further antibodies, derivatives or fragments thereof, which specifically bind the epitope of the invention. Those antibodies may be for instance monoclonal antibodies which can be produced according to usual methods using the epitope as a hapten/component of an antigen.

Moreover the present invention relates to a diagnostic composition containing at least one receptor, preferably at least one monoclonal antibody, fragments or derivatives thereof or aptamers as defined above, optionally fixed to a support material.

Furthermore, the present invention relates to a test device for detecting at least one epitope as defined above, comprising (a) at least one receptor which is preferably a monoclonal antibody, fragments or derivatives thereof or an aptamer as defined above, fixed to a support material; (b) a device for preparing and analysing stool samples; and optionally (c) a mixture of receptors as defined above.

A further subject-matter of the invention is a test device comprising (a) at least one receptor, preferably a monoclonal antibody, fragments or derivatives thereof or an aptamer as defined above, with the receptor being conjugated with colloidal gold, latex particles or other colouring particles, the size of which typically ranges from 5 nm to 100 nm, preferably from 20 nm to 60 nm; (b) a device for preparing and analysing stool samples; and optionally (c) a mixture of receptors as defined above.

Furthermore, the present invention relates to a kit containing (a) at least one receptor which preferably is a monoclonal antibody, fragments or derivatives thereof or an aptamer as defined above and which is optionally fixed to a support material; optionally also (b) a device for preparing and analysing stool samples; and optionally (c) a mixture of receptors as defined above.

The invention also relates to a composition containing at least one of the aforementioned receptors, optionally in combination with a pharmaceutically compatible support and/or a diluent. The composition is preferably a pharmaceutical preparation.

Examples of appropriate pharmaceutically compatible supports are known to the man skilled in the art and include phosphate-buffered saline solutions, water, emulsions such as oil/water emulsions, different kinds of detergents, sterile solutions, etc. Pharmaceutical preparations comprising such supports may be formulated by means of known conventional methods. These pharmaceutical preparations can be administered to an individual in an appropriate dose ranging for example from 1 µg to 100 mg per day and patient. There are various forms of administration, e.g. intravenous, intraperitoneal, subcutaneous, intramuscular, local or intradermal. The doctor in charge of the treatment will choose the kind of dosage in accordance with the clinical factors. The man skilled in the art knows that the kind of dosage depends on various factors such as size, body surface area, age, sex or general state of the patient, but it also depends on the specific pharmaceutical preparation that is administered, the duration and the kind of administration and on other pharmaceutical preparations which are possibly administered at the same time.

Finally, the invention relates to a package containing the diagnostic composition of the invention, the test device of the invention or the kit of the invention.

The components of the diagnostic composition of the invention, the test device of the invention and/or the kit of the invention may be packed in containers such as vials or tubules, optionally in buffers and/or solutions. Possibly, one or more of the components may be packed in one and the same container.

In the Figures:

Figure 1 shows a cloned DNA sequence coding for the V-region of the heavy chain of a monoclonal antibody specific to catalase. The encoded

amino acid sequence is shown in a single-letter code. The CDR regions 1-3 determined according to Kabat et al. are underlined.

Figure 2 shows a cloned DNA sequence coding for the V-region of the light chain of a monoclonal antibody specific to catalase. The encoded amino acid sequence is shown in a single-letter code. The CDR regions 1-3 determined according to Kabat et al. are underlined.

Figure 3 shows the course of an eradication treatment of an *H. pylori*-positive patient after taking Omeprazol, Metronidazol and Clarithromycin.

The examples illustrate the invention.

Example 1: Isolation of *H. pylori* antigens

Cultivation of *H. pylori*

H. pylori (strain NCTC 11637) was plated in petri dishes on Wilkins Chalgren agar adding 10% horse blood and Amphotericin B, Vancomycin and Cefsoludin (Sigma Chemicals) and incubated in a microaerophile atmosphere (Anaerocult GasPAK, Merck) at 37°C for 1-2 days. The content of 2 dishes was suspended in a 1l-bottle (Schott) in 350 ml of BHIB-medium adding the antibiotics as above, the medium was fumigated for 4-8 min with a gas mixture of 10% CO_2, 5% O_2, 85% N_2 and the bottle was sealed. The culture was shaken on a rotary shaker for 2 days at 37°C. Then the content of the bottle was put in a sterile condition in a 10 l-bottle and filled up with 4.7 l of BHIB-medium. It was then incubated on a rotary shaker for another 2 days at 37°C. Subsequently the whole volume was centrifuged at 5,000 g for 15 min, the supernatant was decanted and the bacteria pellet was weighed. In order to store the pellet, it was resuspended in a physiological saline solution with the addition of 15% glycerine at a ratio of 2:1 (w/v) and frozen at –80°C. In order to check the identity of the cultivated bacteria, a microscopic inspection of the bacteria as well as tests for urease, oxidase and catalase activity were carried out.

Example 2: Preparation of *H. pylori* antigens

Preparation of *H. pylori* lysate

H. pylori bacteria pellet (Example 1) was mixed with PBS, pH 7.5 in a ratio of 1:10 and resuspended on ice. The bacteria cells were subjected to ultrasound on ice with a small probe of an ultrasonic device (Sonifer, Branson) with an intensity of 25-30% for 10 x 60 s with a break of 60 s each time. The disrupted bacteria cells were centrifuged 2 x 20 min at 4°C and 10,000 rpm (Sorvall, SS34). The supernatant was used as an antigen preparation for the production of polyclonal antisera.

Preparation of *H. pylori* catalase

Frozen bacteria pellet was mixed with disruption buffer (20 mM Tris HC1, pH 7.0, 1 mM EDTA, 1 mM phenyl methyl sulphonyl fluoride (PMSF), 0.05% sodium azide and 10% (v/v) isobutanol in a ratio of 1:2 (w/v) and shaken at room temperature (RT) in an overhead shaker until complete thawing and subsequently shaken for approximately a further 15 min. After centrifugation at 20,000 rpm (Sorvall, SS-34), 4°C for 20 min, the supernatant was decanted and filtered through a 0.45 µm-filter.

The clear supernatant was diluted with buffer A (20 mM Tris HC1, pH 7.0, 1 mM EDTA, 1 mM PMSF, 0.05% sodium azide) in a ratio of 1:3 and transferred onto a SourceQ column (16/10) (Pharmacia) equilibrated with buffer A. The through-flow of the SourceQ column contained the enzyme catalase and was free of *H. pylori* main antigens such as urease, HSP60 and alkylhydroperoxide reductase.

In order to isolate the catalase, the through-flow of the SourceQ column was subjected to molecular sieve chromatography (Superdex 200) (16/60). The catalase was isolated together with another protein of a size of approx. 150 kDa (neutrophil activating protein, NAP) in about equal shares.

Catalase with a higher purity was obtained when the through-flow of the SourceQ column was brought with a 2 M sodium acetate solution, pH 4.9, to 40 mM sodium acetate and was transferred on a SourceS column (8/28). After washing with a buffer A to remove the proteins that are not

bound, the catalase was eluted with buffer B (40 mM sodium acetate, 1 M NaCl, pH 4.9) using a linear NaCl gradient (buffer A plus 0% to 100% of buffer B). Catalase elutes at approx. 370 mM NaCl.

Example 3: Characterisation of the catalase:

Under reducing conditions in SDA PAGE the purified protein had a molecular weight of approx. 58 kDa and a purity of ≥ 90%.

In order to identify the isolated protein a microsequencing procedure was carried out. The protein was cleaved in SDS PAGE gel with LysC protease. The extracted protein mixture was separated via RP-HPLC. The sequence analysis of the LysC peptide resulted in the following amino acid sequence.

ERLHDTIGESLAHVTHK

This sequence is identical to the corresponding LysC peptide from *H. pylori* catalase (Manos J. et al. (1998) Helicobacter 3 (1), 28-38; Genbank accession No AAC16068.1).

Example 4: Production of polyclonal and monoclonal antibodies (pab; mab)

Production of polyclonal antisera:

Polyclonal antisera against *H. pylori* lysate, *H. pylori* lysate with depleted main antigens such as urease, HSP60 and alkylhydroperoxide reductase (cf. Example 2: isolation and purification), *H. pylori* lysate with enriched catalase (for example by adding catalase to the lysate), as well as polyclonal antisera against purified catalase can be obtained by immunising a selected mammal (e.g. mouse, rabbit, goat, etc.) with the corresponding immunogenic preparations containing catalase epitope.

The antibodies can be purified by means of protein A affinity chromatography from sera and can be used as catching antibodies in sandwich ELISA (cf. Example 9) for assessing the suitability of monoclonal antibodies for antigen detection in the stool of patients.

Polyclonal rabbit antisera were generated by pab Productions (Herbertshausen) from *H. pylori* lysate. By means of protein A affinity chromatography polyclonal antibodies were purified from these antisera

and used as catching antibodies in sandwich ELISA (cf. Example 9) for assessing the suitability of monoclonal antibodies for antigen detection in the stool of patients.

Production of monoclonal antibodies:

The monoclonal antibodies were produced according to methods known to the man skilled in the art (Harlow & Lane, 1988; Peters & Baumgarten, 1990).

Immunisation

Antigen preparations produced from *H. pylori* lysate (cf. Example 2) were used for immunising mice (BALB/c x C57 Black, F1 generation, 8-12 weeks old). For basic immunisation 50 µg antigen were emulsified with Freund complete adjuvant (Difco) in a ratio of 1:1 and injected intraperitoneally (200 µl/mouse). In 4-monthly booster shots the mice were given 25 µg antigen each time with Freund incomplete adjuvant. An antiserum as positive control in ELISA (cf. fusion screening) was obtained from blood taken retro-orbitally from the mice.

Fusion

Two days after the last immunisation the spleens of the mice were removed and the spleen cells were fused with the myeloma cells P3x63Ag8.653 (ATCC CRL-1580; Kearney et al., 1979) with polyethylene glycol 4000 in a ratio of 5:1. The fused cells were suspended in HAT medium (cloning medium (=RPMI 1640 medium, 20% FCS, 200 U/ml rhIL-6) with hypoxanthine aminopterin thymidine supplement (100x concentrate; Sigma)) and plated in 96-well microtitre plates with a cell density of 2-6x10^4 cells/well. The hybridomas were cultivated at 37°C, 5% CO_2, and 95% relative humidity.

Fusion screening by means of direct ELISA

Screening of the antibody-containing culture supernatants from colonised dishes (approx. 10 days after fusion) was carried out in direct ELISA on 96-well microtitre plates (MaxiSorb, Nunc):

The ELISA plates were coated with 2 µg/ml immunisation antigen in carbonate buffer, pH 9.6 (100 µl/well, overnight, 5°C). The coating solution was sucked off and binding sites that were still free were blocked with 2% skimmed-milk powder in PBS (w/v) (200 µl/well, 1 hour, room temperature). After washing the plate twice with PBS, pH 7.3 with 0.025% Tween 20 (v/v), the culture supernatants of the primary clones were pipetted undiluted in the wells (100 µl/well) and the plates were incubated for 1-2 hours at room temperature. Antiserum was used as a positive control, medium as a negative control. After washing again, detection of the bound antibodies was carried out with a peroxidase-labelled secondary antibody (rabbit-anti-mouse Ig-POD (DAKO) in PBS with 0.1% bovine serum albumin, 20 min, room temperature). In the following step the peroxidase turns the colourless substrate tetramethyl benzidine (TMB, Sigma) into a coloured complex. After washing and knocking the plate four times, substrate solution (K-Blue, Neogen or citric acid buffer, pH 4.5, with TMB + H_2O_2) was added and after 10 min the reaction was stopped by adding 1 N sulphuric acid. Culture supernatants of clones producing antigen-specific antibodies were significantly coloured compared to the colourless negative culture supernatants.

Establishing and cultivating the hybridomas

Positive clones were recloned twice using the principle of limit dilution analysis in order to obtain monoclones (Coller & Coller, 1983). The first recloning operation was carried out in cloning medium with hypoxanthine thymidine supplement (100x concentrate; Sigma), the second one in cloning medium. The reclones were in turn examined for antigen specificity by means of direct ELISA. Finally, the final clone was adapted to production medium (RPMI 1640 Medium with 5% IgG-reduced

FCS) in flat bottles. The cells were cryo-preserved and the culture supernatant was produced for antibody purification.

Example 5: Characterisation of the antibodies from culture supernatant

10 clones were selected from a repertory of 30 specific (producing antibodies against the immunisation antigen) clones on the basis of good reactivity to stool samples of patients infected with *H. pylori* in sandwich ELISA (cf. Table 2).

Isotyping

In the culture supernatant isotyping of the monoclonal antibody was carried out with the established clones using the isotyping kit IsoStrip (Roche Diagnostics). That gave 8 type IgG1-clones and one type IgG2a-clone (cf. Table 3).

Western blot

In Western blot the culture supernatants were examined for their ability to specifically recognise the immunising antigen. 15 µg of purified antigen per gel was boiled in reducing sample buffer (Laemmli, 1970) and applied to a 12%-SDS polyacrylamide minigel (8.6 cm x 7.7 cm x 0.1 cm, Biometra). After electrophoretic separation at 25-30 mA the proteins (antigen) were immobilised on a nitrocellulose membrane by means of a semi-dry blot method.

The membrane was blocked with 2% skimmed-milk powder in PBS (30 min, room temperature) and washed three times for 5 mins with TBS/Tween 20 (0.2%). For the following incubation step the membrane was clamped in an Accutran cross blot screening unit (Schleicher and Schüll) using a grid plate with 34 cross channels. In each of the traces that were formed 250 µl of TBS/Tween 20 was provided and in each case 250 µl of the hybridoma culture supernatants to be tested added. Incubation was carried out with shaking for 2 h at room temperature.

After washing three times with TBS/Tween 20, the membrane was incubated for 1 h with the POD-conjugated secondary antibody (rabbit-anti-mouse Ig POD, DAKO). The membrane was washed three times and the

immune complex was visualised by adding the 3,3-diaminobenzidine substrate solution (DAB, Sigma). The antibody-binding protein bands were subsequently visualised by an insoluble peroxidase substrate.

6 hybridoma culture supernatants exhibited a band that corresponds to the catalase (58 kDa), 3 were negative in Western blot but exhibited a positive reaction with native antigen in ELISA. They are likely to recognise a conformation epitope. Table 3 shows a summary of results.

Example 6: Purification of monoclonal antibodies from hybridoma culture supernatants

The purification of mab from serum-free hybridoma culture supernatants is carried out by means of modified protein-G affinity chromatography (Pharmacia Biotech, 1994). The filtered (0.45 µm) culture supernatants were conducted directly over a protein G matrix. Protein detection in the through-flow or in the eluate was carried out by way of measuring the optical density at 280 nm. After washing with 150 mM PBS, pH 7.2, until the detector background value was attained, elution was conducted with 0.1 M glycine/HCl, pH 3.3. The protein G matrix was regenerated with 0.1 M glycine/HCl, pH 2.7.

Example 7: Production of conjugates

Coupling of mab to biotin for use in ELISA

After purification the monoclonal antibodies are biotinylated so that they can be used as detection antibodies in ELISA. Coupling of monoclonal antibodies to biotin and POD was carried out according to known methods (Harlow & Lane, 1988).

The monoclonal antibodies were conjugated at a concentration of approx. 1-2 mg/ml. Before coupling the antibodies were rebuffered by dialysis in 0.1 M sodium acetate buffer, pH 8.3 or 0.1 M sodium hydrogen carbonate buffer, pH 8.3. For each 1 mg of antibodies 50 µg N-hydroxysuccinimidobiotin (NHS-d-biotin; Sigma) was pipetted and mixed in DMSO. The mixture was incubated for one hour at room temperature. Then the biotinylated antibodies were freed of uncoupled NHS-d-biotin by extensive dialysis against 0.15 M PBS, 0.05% NaN_3, pH 7.5.

Coupling of mab to colloidal gold for use in immunological rapid tests

The monoclonal antibody (mab) was conjugated to colloidal gold for use in immunological rapid tests. This is carried out according to known standard methods (Frens, 1973; Geoghegan and Ackerman, 1977; Slot et al., 1985). For the production of colloidal gold for example 200 ml of a 0.01% gold chloride-($HAuCl_4$)-solution is heated until boiling and reduced with further boiling by adding 2 ml of 1% sodium citrate ($Na_3C_6H_5O_7$). Control of the particle size is effected by way of the concentration of sodium citrate. During a reaction time of 5 – 20 min, colloid formation is effected with a characteristic colour change to wine-red. After cooling of the gold solution the pH-value can be adjusted by means of 0.2 M K_2CO_3 or 0.1 M HCl to the desired pH-value for IgG coupling.

For coupling mab to colloidal gold an amount of IgG necessary for stabilisation is mixed with the gold solution and incubated for 15 min at room temperature. The optimum IgG concentration and the suitable pH-value for coupling have to be determined individually for each mab. 10 µg IgG/ml of gold colloid can be used as a guideline. Polymers or protein, e.g. bovine serum albumin (BSA), are added in a concentration of 1% to the coupling preparation in order to stabilise the gold IgG conjugate. Gold colloid that was not coupled to IgG and free IgG are subsequently removed from the coupling preparation by centrifugation of the gold IgG conjugate. The gold IgG conjugate forms a loose sediment which is coloured dark red and which can be separated from the clear supernatant by suction removal. For storage, preferably at 4°C, 0.05% of NaN_3 can be added to the solution buffer of the gold IgG conjugate.

Example 8: Characterisation of the purified monoclonal antibody

Characterisation of antibody-antigen interactions by means of surface plasmon resonance spectroscopy (SPR spectroscopy)

By means of SPR spectroscopy it is possible to determine the affinity constants of the monoclonal antibodies. Suitable antibodies for the development of ELISA and rapid test can be found in that way.

Conducting surface plasmon resonance spectroscopy on the Pharmacia BIAcore

All steps were carried out on a Pharmacia Biacore Processing Unit CA 186 according to the manufacturer's instructions (BIAcore Methods Manual).

Catalase was immobilized by way of amine coupling on the dextrane matrix of the BIAcore CM5 sensor chip. For activation of the dextrane matrix 45 µl of a 1:1 mixture of 0.05 M N-hydroxysuccinimide (NHS) and 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) solution was passed over the sensor chip at a flow rate of 5 µl/min. Then catalase (35 µl; 50 µg/ml in a 10 mM sodium acetate pH 5.0) was bound to the dextrane matrix. Remaining NHS esters were deactivated with 1 M ethanolamine (35 µl). Catalase that was not covalently bound to the dextrane matrix was removed by regenerating the sensor chip with HCl (10 mM; 15 µl).

By adding the catalase-specific monoclonal antibodies, they were made to react with immobilised catalase and the mass attachment to the detector was measured. Antibody solutions in different concentrations ranging from 20 to 670 nM were used. They were injected over the catalase immobilised on the sensor chip CM5 at a flow rate of 25 µl/min in each case.

Results:

The values for the rate constants of adsorption (k_{on}) and desorption (k_{off}) of the antibody can be calculated from the time course of the resonance signal (BIAevaluation software 3.0). 6 monoclonal antibodies against catalase were tested as to their affinities:

Table 1: Results of affinity determination of catalase-mab

Mab	$k_{on}[M^{-1} s^{-1}]$	$k_{off}[s^{-1}]$	$K_D[M]$
HP25.2m/2h10	1.44E+05	3.90E-05	2.71E-10
HP25.6m/1G4	1.41E+05	2.52E-05	1.79E-10
HP25.6m/1B5	5.67E+04	3.86E-05	6.81E-10
HP25.6m/4E3	4.92E+04	5.96E-05	1.21E-09
HP25.6m/1A5	3.91E+04	4.77E-05	1.22E-09
HP25.6m/1H4	7.12E+04	4.12E-05	5.79E-10

$K_D = k_{off} : k_{on}$

Selection of antibody pairings for use in ELISA on human stool

These antibodies which exhibited the lowest detection limits when the culture supernatant was measured were determined by means of surface plasmon resonance epitope overlaps and affinity constants were measured. The combinations which were promising in these measurements (no epitope overlaps, high rate constant for adsorption, low rate constant for desorption) were tested for their antigen detection limit in sandwich stool ELISA.

Example 9: Screening of mab culture supernatants on patient samples (mixed polyclonal/monoclonal system)

Those monoclonal antibodies were analysed as culture supernatants in sandwich ELISA as to their patient recognition and antigen detection limit.

As internal development samples, stool samples were available, the infection status of which (groups 0 and 4) was determined by means of histological investigation and/or ^{13}C urea breath test. In the case of patients of group 0 an infection with *H. pylori* could be reliably excluded, in the case of patients of group 4 an infection could be certainly detected.

The ELISA plates (MaxiSorb; Nunc) were coated overnight at 5°C with 100 µl of a solution of a polyclonal rabbit-anti-catalase antibody or polyclonal rabbit-anti-*H. pylori* antibody (pab; approx. 20 µg IgG/ml 0.1 M carbonate buffer, pH 9.5). In order to block the binding sites that were still

free, 200 µl of 150 mM PBS pH 7.2 with 0.2% fish gelatine (w/v) was pipetted per well and incubated at room temperature for 30 min. Then the ELISA plate was washed twice with 250 µl PBS adding 0.025% Tween 20 (washing buffer 1). Human stool was suspended with 150 mM in a ratio of 1:10 (w/v) with the addition of 2% skimmed-milk powder and 1 mM EDTA.

For determining the antigen detection limit an *H. pylori*-negative stool suspension was mixed with 50 ng/ml catalase (cf. Example 2) and diluted with an *H. pylori*-negative stool suspension in 1:2 steps. 100 µl of the stool suspension per well was incubated for one hour (double determination in the case of patient samples). The plate was knocked out, rinsed with washing buffer 2 PBS with 0.2% Tween 20 and washed 4 times with washing buffer 2. Then, 100 µl culture supernatant of hybridomas (1:5 diluted in PBS) was added and incubated at room temperature for 60 min. The bound antibodies are detected by adding a conjugated secondary antibody (rabbit-anti-mouse IgG-POD, DAKO). In the next step the POD turns the colourless substrate tetramethylbenzidine (TMB, Sigma) into a blue product. After 5 to 10 minutes, or as soon as the negative control exhibited a light blue coloration, the enzyme reaction was stopped by adding 1 N sulphuric acid (100 µl/well). The intensity of the colour reaction was measured in the ELISA reader (MWG Spektral). Measurement is carried out at 450 nm against the reference wavelength of 620 nm. Before the detection antibody or the substrate solution was added, the ELISA plate was washed three to four times in each case with washing buffer 1.

The lowest concentration at which extinction greater than or equal to double the control (*H. pylori*-negative stool sample without antigen addition) was still detected was determined to serve as the detection limit.

Table 2: HP25.2m/2H10: sensitivity and specificity in sandwich ELISA with patient samples

stool sample	patient infection status	catching ab: pab against HP detection ab: HP25.2m/2H10 (culture supernatant) $OD_{450-620}$	evaluation cut off: 0.1: $OD_{450-620}$ =0.1
CX0010	POSITIVE	0.25	positive
CX1014	POSITIVE	0.75	positive
CX1029	POSITIVE	0.18	positive
CX1038	POSITIVE	0.09	negative
CX1052	POSITIVE	0.11	positive
CX2008	POSITIVE	0.63	positive
CX2009	POSITIVE	0.32	positive
CX2016	POSITIVE	0.07	negative
CX2019	POSITIVE	0.59	positive
CX2029	POSITIVE	0.52	positive
CX2013	POSITIVE	0.04	negative
CX294-1	POSITIVE	0.14	positive
CX3098	POSITIVE	0.13	positive
CX3146	POSITIVE	0.05	negative
CX3148	POSITIVE	0.08	negative
CX3234	POSITIVE	0.18	positive
CX4003	POSITIVE	0.17	positive
CX4006	POSITIVE	0.25	positive
CXT001	POSITIVE	0.23	positive
CXT002	POSITIVE	0.53	positive
CXT003	POSITIVE	0.12	positive
CXT004	POSITIVE	0.03	negative
CXT005	POSITIVE	0.03	negative
CXT006	POSITIVE	0.31	positive
CXT007	POSITIVE	0.08	negative
CX1008	NEGATIVE	0.29	positive
CX1031	NEGATIVE	0.08	negative

CX1049	NEGATIVE	0.7	positive
CX1051	NEGATIVE	0.09	negative
CX0142	NEGATIVE	0.03	negative
CX0185	NEGATIVE	0.03	negative
CX0189	NEGATIVE	0.08	negative
CX0193	NEGATIVE	0.03	negative
CX2010	NEGATIVE	0.08	negative
CX2018	NEGATIVE	0.09	negative
CX0220	NEGATIVE	0.03	negative
CX0231	NEGATIVE	0.03	negative
CX0258	NEGATIVE	0.02	negative
CX3008	NEGATIVE	0.09	positive
CX3011	NEGATIVE	0.08	negative
CX3033	NEGATIVE	0.07	negative
CX3035	NEGATIVE	0.09	negative

Abbreviations pab: polyclonal antibody; HP: *H. pylori*

The monoclonal antibody HP25.2m/2H10 exhibits in the sandwich ELISA with patient samples a sensitivity of 68% (out of 25 positive samples 17 were detected) and a specificity of 82% (out of 17 samples 14 were correctly detected).

Table 3: Characterisation of the monoclonal antibodies against catalase

fusion/clone	isotype	WB (ag)	NWG (ng/ml)	stool samples that were correctly detected	
				pos.samples	neg.samples
HP25.2m/2H10	IgG2a, K	+	1.5	17 out of 25	14 out of 17
HP25.6m/IG4	IgG1, K	+	1.5	4 out of 5	2 out of 2
HP25.6m/IB5	IgG1, K	+	3-6	3 out of 5	2 out of 2
HP25.6m/IH4	IgG1, K	+	3-6	2 out of 5	2 out of 2
HP25.6m/4E3	IgG1, K	+	6	2 out of 5	2 out of 2
HP25.6m/1A5	IgG1, K	+	6	2 out of 5	2 out of 2

HP25.6m/5E4	IgG1, K	-	1.5	1 out of 5	2 out of 2
HP25.6m/4A12	IgG1, K	-	1.5	1 out of 5	2 out of 2
HP25.6m/5F4	IgG1, K	-	1.5	1 out of 5	2 out of 2

Abbreviations: ag: antigen; WB: Western blot; NWG: detection limit

Results:

Table 3 summarises the results of isotype determination, Western blot analyses, determination of detection limits and patient recognition for the monoclonal antibodies against catalase. The data show that good detection of native catalase by means of mab does not correlate with good patient recognition.

In the mixed polyclonal/monoclonal sandwich ELISA system the mab HP25.2m/2H10 exhibited a sensitivity of 68% and a specificity of 82%. An improvement in sensitivity and specificity was exhibited by using purified mab (instead of culture supernatant) in a purely monoclonal ELISA system. In this case, either a monoclonal antibody directed against the same epitope of the antigen or two different monoclonal antibodies directed against different epitopes of the same antigen (cf. Example 10) can be used as catching and detection antibodies.

Example 10: Detection of *H. pylori* in human stool by means of ELISA (purely monoclonal system)

For the test stool samples of patients of ten different hospitals or gastroenterological surgeries were available, whose *H. pylori*-negative or *H. pylori*-positive was determined by means of ^{13}C urea breath test and/or histological investigations of gastric biopsies. The stool samples to be tested were coded so that the laboratory staff did not know the infection status.

***H. pylori* stool sandwich ELISA**

The ELISA plates (MaxiSorb; Nunc) were coated with 100 µl of a mab solution (2.0 µg HP25.2m/2H10/ml, 0.1 M carbonate buffer, pH 9.5) for 1 hour at 37°C. In order to block the binding sites that were still free, 200 µl 150 mM PBS with 0.2% fish gelatine (w/v) was pipetted per dish and

incubated at room temperature for 30 min. Subsequently they were washed twice with 250 µl washing buffer 1 (PBS with 0.025% Tween). Human stool was suspended with 150 mM PBS in a ratio of 1:10 (w/v) with the addition of 2% of skimmed-milk powder and 1 mM EDTA. In order to determine the antigen detection limit purified *H. pylori* catalase was added in known concentrations to the stool suspension of an *H. pylori*-negative patient. The stool sample suspensions were centrifuged off at 7,000 g for 5 min. 100 µl of the supernatant per well was incubated for one hour. The plate was knocked off, rinsed and washed four times with washing buffer 2 (250 µl PBS with the addition of 0.2% Tween). Then 100 µl of a solution of biotin-coupled mab (1 µg/ml HP25.2m/2H10-Bio in PBS; 0.1% BSA) was added and incubated at room temperature for 60 min. The bound antibodies are detected by adding a conjugate of streptavidin with POD (Dianova). In the next step the POD then turns the colourless substrate TMB (Sigma) into a blue product. After five to ten minutes or as soon as the negative control exhibited a light blue coloration the reaction was stopped by adding 1N sulphuric acid (100 µl/well). The intensity of the colour reaction was measured in the ELISA reader (MWG Spektral). Measurement is carried out at 455 nm against the reference wavelength of 620 nm.

Result:

Table 4: Detection of *H. pylori* catalase in the stool by means of ELISA using the monoclonal antibody HP25.2m/2H10

patients	clinical status	HP stool ELISA OD (455-620)
1001	negative	0.069
1002	negative	0.104
1007	negative	0.053
1008	negative	0.042
1010	negative	0.043
1012	negative	0.55
1017	negative	0.052
1021	negative	0.045
1022	negative	0.068
1024	negative	0.036
1025	negative	0.046
1027	negative	0.057
1030	negative	0.061
1031	negative	0.037
1032	negative	0.056
1034	negative	0.048

1035	negative	0.033
1040	negative	0.037
1046	negative	0.046
2002	negative	0.056
2006	negative	0.032
2007	negative	0.027
2010	negative	0.039
2012	negative	0.041
2013	negative	0.049
2014	negative	0.046
2015	negative	0.048
2017	negative	0.050
2018	negative	0.061
2023	negative	0.056
2024	negative	0.051
2028	negative	0.102
2033	negative	0.050
2034	negative	0.077
2043	negative	0.045
3123	negative	0.055
3213	negative	0.119
3214	negative	0.062
3224	negative	0.048
3225	negative	0.065
3236	negative	0.043
4004	negative	0.089
5004	negative	0.079
5007	negative	0.055
5008	negative	0.156
5009	negative	0.076
5010	negative	0.073

5012	negative	0.051
5013	negative	0.057
5017	negative	0.064
5018	negative	0.033
5019	negative	0.017
5020	negative	0.017
5021	negative	0.019
5022	negative	0.020
5024	negative	0.015
5025	negative	0.017
5027	negative	0.022
5028	negative	0.021
5030	negative	0.019
5031	negative	0.014
5033	negative	0.018
5034	negative	0.013
5035	negative	0.018
5036	negative	0.031
5040	negative	0.024
5042	negative	0.026
5046	negative	0.021
5052	negative	0.020
5056	negative	0.523
5057	negative	0.023
5060	negative	0.055
5063	negative	0.022
5064	negative	0.017
5065	negative	0.035
5066	negative	0.024
5067	negative	0.088
5068	negative	0.021

6002	negative	0.078
6005	negative	0.019
6008	negative	0.013
6019	negative	0.034
7005	negative	0.025
7006	negative	4.556
7009	negative	0.030
7013	negative	0.024
8004	negative	0.023
8047	negative	0.021
213	positive	0.879
294	positive	4.097
444-1	positive	0.201
1003	positive	0.475
1013	positive	4.087
1014	positive	0.105
1015	positive	2.469
1028	positive	0.096
1029	positive	4.466
1037	positive	2.485
2001	positive	0.083
2003	positive	0.817
2005	positive	1.508
2008	positive	4.247
2009	positive	1.597
2016	positive	2.651
2022	positive	0.135
2029	positive	3.953
2032	positive	3.400
2035	positive	3.384
2039	positive	0.053

2040	positive	4.602
2041	positive	0.200
2042	positive	4.592
3146	positive	1.742
6014	positive	2.572
3149	positive	0.989
3153	positive	4.590
3570	positive	4.567
3577	positive	4.566
3215	positive	4.540
3219	positive	4.486
3220	positive	4.518
3231	positive	4.706
3234	positive	4.567
3235	positive	4.616
5069	positive	1.079
CXT 5	positive	0.602
5072	positive	4.151
5075	positive	4.307
5076	positive	4.516
CXT 4	positive	0.268
5078	positive	1.022
6001	positive	4.441
6004	positive	4.296
CXT 3	positive	2.126
3241	positive	3.671
3243	positive	4.582
4003	positive	4.700
4005	positive	0.401
4006	positive	4.694
4018	positive	4.142

4019	positive	2.366
4020	positive	1.468
5001	positive	4.490
5002	positive	3.917
5003	positive	4.321
5006	positive	4.826
77	positive	0.067
5011	positive	0.071
53	positive	4.773
70	positive	1.084
5016	positive	0.101
68	positive	4.611
67	positive	0.589
5029	positive	0.675
64	positive	1.785
58	positive	0.304
5039	positive	3.391
CXT 13	positive	3.785
6013	positive	1.972
CXT 12	positive	0.157
5048	positive	1.695

5050	positive	0.490
CXT 10	positive	0.247
5053	positive	4.232
5055	positive	4.364
CXT 9	positive	2.455
5058	positive	3.886
5059	positive	4.450
CXT 8	positive	4.374
5061	positive	4.032
CXT 7	positive	0.647
CXT 6	positive	4.592
6018	positive	4.656
6020	positive	0.427
7001	positive	2.717
CXT 2	positive	4.479
7002	positive	4.143
7003	positive	0.149
7004	positive	4.543
CXT 1	positive	0.953
8026	positive	0.025
8033	positive	0.784

H. pylori-ELISA (n = 181)

		H. pylori infection status	
		positive	negative
H. pylori stool sandwich ELISA	positive	88	6
cut off $OD_{450-620}$: 0.09	negative	5	82

Sensitivity: 94.6%

Specificity: 93.2%

Table 4 shows the results of the investigation of *H. pylori*-negative and *H. pylori*-positive stool samples by means of stool sandwich ELISA. In this case monoclonal antibodies are used for detection of the *H. pylori* antigen catalase from the stool sample. The catalase is an extremely stable antigen which passes through the digestive tract substantially unchanged and can thus be detected in the stool sample. The investigation of 181 stool samples in the purely monoclonal ELISA system, which is based on only one catalase-specific mab, has a sensitivity of 94.4% and a specificity of 93.2%. That sensitivity and specificity leads to such high positive and negative predictive values that it is possible to detect an infection with *H. pylori* with sufficient reliability by simple, inexpensive and non-invasive stool analysis in order to decide on an eradication treatment. Sensitivity and specificity can possibly be increased by a combination of different mabs which are directed against different epitopes of the catalase or by a combination of two detection systems for different antigens (e.g. catalase/urease).

Example 11: Course of eradication

Eradication control can only be carried out by way of direct detection of *H. pylori* antigens and not of antigens in serum since after an infection *H. pylori* antibodies are still present in the blood for many months. Thus in contrast to serologic *H. pylori* tests the described sandwich stool ELISA offers the possibility of assessing the success of an eradication. Figure 3 shows the course of an eradication treatment of an *H. pylori*-positive patient after application of Omeprazol, Metronidazol and Clarithromycin. 6 days after beginning the therapy no more *H. pylori* antigen could be detected in the stool.

Example 12: Cloning and sequence determination of the functional variable regions of immunoglobulins from hybridoma cell lines

Total RNA was isolated from antibody-producing hybridoma cell lines according to Chomczynski (Chomczynski. 1987).

Then the corresponding cDNA was synthesised using standard methods (Sambrook et al., 1989).

The DNA regions encoding the kappa-light chain as well as the heavy chain Fd segment (VH or CH1) of the respective antibodies were amplified by means of PCR. The oligonucleotide primer set stated in Table 10 was used, the cDNA isolated from the single hybridoma cell lines served as a template.

The primer set used leads to a 5'-*XhoI* and a 3'-*SpeI* cleavage site in the heavy chain Fd fragments as well as to a 5'-*SacI* and a 3'-*XbaI* cleavage site in the kappa-light chains. For PCR amplification of the DNA fragments encoding the heavy chain Fd, 11 different 5'-VH primers (MVH 1-8 and MULH 1-3) were each combined with the 3'-VH primer MIgG2a. For the amplification of the DNA fragments which encode the kappa-light chains, 11 different 5'-VK primers (MUVK 1-7 and MULK 1-4) were each combined with the 3'-VK primer 3' MUCK.

The following temperature program was used in all PCR amplification procedures: denaturation at 94°C for 30 s, primer attachment at 52°C for 60 s, polymerisation at 72°C for 90 s. This program was maintained for 40 cycles, followed by a final completion of the fragments at 72°C for 10 min.

The results of the PCR amplification procedures were separated by means of agarose gel electrophoresis and DNA bands of the expected molecular weight were isolated. The isolated bands were then subjected to restriction digestion using the enzymes XhoI and SpeI (heavy chains), and SacI and XbaI (light chains) respectively and the fragments obtained were cloned into the plasmid vector Bluescript KS (Strategene) after the vector had first been cleaved with the restriction enzymes XhoI and SpeI, and SacI and XbaI respectively.

Subsequently, plasmid preparations of the cloned heavy and light chain fragments were sequence-analysed. Sequences were chosen which encode the functional variable regions of the heavy and light chains of immunoglobulin (VH or VL). In that way it was possible to identify exactly one functional VH and one functional VL region for each hybridoma cell line. Figure 1 and Figure 2 show the functional VH and VL sequences. The first four amino acids of the VH region were completed by recloning. Cloning

and sequencing were carried out using standard methods (Sambrook et al., 1989).

Table 5: List of the primers used for the PCR amplification of the functional variable regions of heavy and light immunoglobulin-chains (orientation 5'-3')

MVH1	(GC)AG GTG CAG CTC GAG GAG TCA GGA CCT
MVH2	GAG GTC CAG CTC GAG CAG TCT GGA CCT
MVH3	CAG GTC CAA CTC GAG CAG CCT GGG GCT
MVH4	GAG GTT CAG CTC GAG CAG TCT GGG GCA
MVH5	GA(AG) GTG AAG CTC GAG GAG TCT GGA GGA
MVH6	GAG GTG AAG CTT CTC GAG TCT GGA GGT
MVH7	GAA GTG AAG CTC GAG GAG TCT GGG GGA
MVH8	GAG GTT CAG CTC GAG CAG TCT GGA GCT
MULK1	GGG GAG CTC CAC CAT GGA GAC AGA CAC ACT CCT GCT AT
MULK2	GGG GAG CTC CAC CAT GGA TTT TCA AGT GCA GAT TTT CAG
MULK3	GGG GAG CTC CAC CAT GGA GWC ACA KWC TCA GGT CTT TRT A
MULK4	GGG GAG CTC CAC CAT GKC CCC WRC TCA GYT YCT KGT
MIgG2a	GAG AGA GGG GTT CTG ACT AGT GGG CAC TCT GGG CTC
MUVK1	CCA GTT CCG AGC TCG TTG TGA CTC AGG AAT CT
MUVK2	CCA GTT CCG AGC TCG TGT TGA CGC AGC CGC CC
MUVK3	CCA GTT CCG AGC TCG TGC TCA CCC AGT CTC CA
MUVK4	CCA GTT CCG AGC TCC AGA TGA CCC AGT CTC CA
MUVK5	CCA GAT GTG AGC TCG TGA TGA CCC AGA CTC CA
MUVK6	CCA GAT GTG AGC TCG TCA TGA CCC AGT CTC CA
MUVK7	CCA GTT CCG AGC TCG TGA TGA CAC AGT CTC CA
MULH1	GGG CTC GAG CAC CAT GGR ATG SAG CTG KGT MAT SCT CTT
MULH2	GGG CTC GAG CAC CAT GRA CTT CGG GYT GAG CTK GGT TTT
MULH3	GGG CTC GAG CAC CAT GGC TGT CTT GGG GCT GCT CTT CT
3'MUCK	GCG CCG TCT AGA ATT AAC ACT CAT TCC TGT TGA A

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CLAIMS

1. A method of detecting an infection of a mammal with an acid-resistant microorganism, wherein

(a) a stool sample of the mammal is incubated with (aa) a receptor under conditions permitting a complex formation of an antigen from the acid-resistant microorganism with the receptor; or (ab) two different receptors under conditions permitting a complex formation of an antigen from the acid-resistant microorganism with the two receptors, and wherein the receptor according to (aa) or the receptors according to (ab) specifically bind(s) an antigen which at least in some of the mammals has a structure after passage through the intestine that corresponds to the native structure or the structure against which a mammal produces antibodies after being infected or immunised with the acid-resistant microorganism or an extract or lysate thereof or a protein therefrom or a fragment thereof or a synthetic peptide; and

(b) the formation of at least one antigen-receptor complex according to (a) is detected.

2. A method according to claim 1 wherein the microorganism is an acid-resistant bacterium.

3. A method according to claim 2 wherein the acid-resistant bacterium is a bacterium of the genus Helicobacter, Campylobacter or the genus Mycobacterium.

4. A method according to claim 3 wherein the bacterium is a bacterium of the species *Helicobacter pylori, Helicobacter hepaticus, Campylobacter jejuni* or *Mycobacterium tuberculosis*.

5. A method according to one of claims 1 to 4 wherein the antigen is an antigen of a catalase, preferably of *H. pylori*.

6. A method according to one of claims 1 to 5 wherein the receptor/receptors is/are an antibody or antibodies, a fragment or fragments or derivative or derivatives thereof or an aptamer or aptamers.

7. A method according to one of claims 1 to 6 wherein a mixture of receptors is additionally used for the detection operation, wherein the mixture of receptors functions as a catcher of the antigen if the receptor is used as a detector of the antigen and the mixture functions as a detector of the antigen if the receptor is used as a catcher of the antigen.

8. A method according to claim 7 wherein the mixture of receptors is a polyclonal antiserum.

9. A method according to claim 8 wherein the polyclonal antiserum against a lysate of the microorganism was obtained.

10. A method according to claim 9 wherein the lysate is a lysate with an enriched antigen.

11. A method according to claim 9 or claim 10 wherein the lysate is a lysate with depleted immunodominant antigens.

12. A method according to claim 8 wherein the polyclonal antiserum against a purified or a (semi)synthetically produced antigen was obtained.

13. A method according to claim 12 wherein the antigen is an antigen of a catalase.

14. A method according to one of claims 1 to 13 wherein the receptor and/or the mixture of receptors binds/bind a confirmation epitope or epitopes.

15. A method according to one of claims 6 to 14 wherein the heavy chain of the antibody binding a catalase epitope has at least one of the following CDRs, preferably the CDR3 and more preferably all of the following three CDRs:

CDR1: NYWIH

CDR2: YINPATGSTSYNQDFQD

CDR3: EGYDGFDS

16. A method according to claim 15 wherein the DNA sequence encoding the heavy chain of the antibody has at least one of the following CDRs, preferably the CDR3 and more preferably all of the following three CDRs:

CDR1: AACTACTGGA TTCAC

CDR2: TACATTAATC CTGCCACTGG TTCCACTTCT TACAATCAGG ACTTTCAGGA C

CDR3: GAGGGGTACG ACGGGTTTGA CTCC

17. A method according to one of claims 6 to 16 wherein the light chain of the antibody binding a catalase epitope has at least one of the following CDRs, preferably the CDR3 and more preferably all of the following three CDRs:

CDR1: SASSSVNYMY

CDR2: DTSKLAS

CDR3: QQWSSNPYT

18. A method according to claim 17 wherein the DNA sequence encoding the light chain of the antibody has at least one of the following CDRs, preferably the CDR3 and more preferably all of the following three CDRs:

CDR1: AGTGCCAGCT CAAGTGTAAA TTACATGTAC

CDR2: GACACATCCA AATTGGCTTC T

CDR3: CAGCAGTGGA GTAGTAATCC GTACACG

19. A method according to one of claims 6 to 18 wherein the antibodies in the variable regions of the light and heavy chains have the amino acid sequences shown in Figures 1 and 2.

20. A method according to one of claims 6 to 19 wherein the encoding regions of the variable regions of the light and heavy chains have the DNA sequences shown in Figures 1 and 2.

21. A method according to one of claims 1 to 20 wherein the following steps are carried out with the stool sample prior to incubation with the antibodies: (a) the stool sample is resuspended in a resuspension buffer in a ratio of 1:3 to 1:25, preferably about 1:10, and (b) mixing on a vortex mixer is then effected.

22. A method according to one of claims 1 to 21 wherein detection of the formation of the at least one antigen-receptor complex/antigen-receptor-receptor-mixture complex in step (b) is effected by means of an immunological method.

23. A method according to one of claims 1 to 22 wherein detection of the formation of the at least one antigen-receptor complex/antigen-receptor-receptor-mixture complex in step (b) is effected by means of ELISA, RIA, Western Blot or an immunochromatographic method.

24. A method according to claim 22 or 23 wherein the same receptor is used in the RIA or ELISA for binding to the solid phase as for detection of the epitope.

25. A method according to one of claims 1 to 24 wherein the receptor is fixed to a support.

26. A method according to one of claims 1 to 36 wherein the receptor is a monoclonal murine antibody.

27. A method according to claim 25 wherein the support material of the support is a porous support material.

28. A method according to claim 25 or 26 wherein the support material is a test strip.

29. A method according to claim 25, claim 27 or claim 28 wherein the support material comprises cellulose or a cellulose derivative.

30. A method according to one of claims 1 to 29 wherein the mammal is a human being.

31. A monoclonal antibody, fragment or derivative thereof which has a V-region which has a combination of the CDRs set forth in one of claims 15 to 18.

32. A monoclonal antibody, fragment or derivative thereof according to claim 31 which has at least one of the V-regions shown in Figures 1 and 2.

33. A monoclonal antibody, fragment or derivative thereof according to claim 31 and claim 32 which is a murine antibody or a fragment or derivative thereof or a chimeric, preferably humanised antibody or a fragment or derivative thereof.

34. An aptamer which specifically binds the same epitope as the monoclonal antibody, the fragment or derivative thereof according to one of claims 31 to 33.

35. An epitope which is specifically bound by a monoclonal antibody, fragment or derivative thereof according to one of claims 31 to 33 or the aptamer according to claim 34.

36. An antibody, fragment or derivative thereof which specifically binds an epitope according to claim 35.

37. A diagnostic composition containing at least one receptor as defined in one of the preceding claims, optionally fixed to a support material, which optionally also contains a mixture of receptors as defined in one of the preceding claims, optionally fixed to a support material.

38. A test device for detecting at least one epitope as defined in one of the preceding claims comprising

(a) at least one receptor as defined in one of the preceding claims, fixed to a support material; and optionally

(b) a device for preparing and analysing stool samples; and optionally

(c) a mixture of receptors as defined in one of the preceding claims.

39. A test device for detecting at least one epitope as defined in one of the preceding claims comprising

(a) at least one receptor as defined in one of the preceding claims, wherein the receptor is conjugated with colloidal gold, latex particles or other colouring particles, the size of which is typically in the range of between 5 nm and 100 nm, preferably between 20 nm and 60 nm;

(b) a device for preparing and analysing stool samples; and optionally

(c) a mixture of receptors as defined in one of the preceding claims.

40. A kit containing

(a) at least one receptor as defined in one of the preceding claims, optionally fixed to a support material; optionally also

(b) a device for preparing and analysing stool samples; and optionally

(c) a mixture of receptors as defined in one of the preceding claims.

41. A composition, preferably a pharmaceutical preparation, containing at least one of the above-described receptors, optionally in combination with a pharmaceutically compatible support and/or diluent.

42. A package containing the diagnostic composition according to claim 37, the test device according to claim 38, 39 or the kit according to claim 40.

Abstract

The invention concerns a method of detecting an infection of a mammal with an acid-resistant microorganism, wherein (a) a stool sample of the mammal is incubated with (aa) a receptor under conditions permitting a complex formation of an antigen from the acid-resistant microorganism with the receptor; or (ab) two different receptors under conditions permitting a complex formation of an antigen from the acid-resistant microorganism with the two receptors, and wherein the receptor according to (aa) or the receptors according to (ab) specifically bind(s) an antigen which at least in some of the mammals has a structure after passage through the intestine that corresponds to the native structure or the structure against which a mammal produces antibodies after being infected or immunised with the acid-resistant microorganism or an extract or lysate thereof or a protein therefrom or a fragment thereof or a synthetic peptide; and (b) the formation of at least one antigen-receptor complex according to (a) is detected. Preferably the acid-resistant microorganism is a bacterium, in particular *Helicobacter pylori, Helicobacter hepaticus, Campylobacter jejuni* or *Mycobacterium tuberculosis*. It is further preferred that the receptor/receptors binds/bind to an epitope/epitopes of a catalase. The invention further concerns diagnostic and pharmaceutical compositions and test devices which contain the aforesaid components and packages containing same.

Fig. 1

```
  E   V   Q   L   L   E   Q   P   G   A
GAGGTGCAGC TGCTCGAGCA GCCTGGGGCT  30

  E   L   A   K   P   G   A   S   V   K
GAACTGGCAA AACCTGGGGC CTCAGTGAAG  60

  M   S   C   K   A   S   G   Y   T   F
ATGTCCTGCA AGGCTTCTGG CTACACCTTT  90

  T   N   Y   W   I   H   W   V   K   Q
ACTAACTACT GGATTCACTG GGTGAAACAG 120

  R   P   G   Q   G   L   K   W   I   G
AGGCCTGGAC AGGGTCTGAA ATGGATTGGA 150

  Y   I   N   P   A   T   G   S   T   S
TACATTAATC CTGCCACTGG TTCCACTTCT 180

  Y   N   Q   D   F   Q   D   R   A   T
TACAATCAGG ACTTTCAGGA CAGGGCCACT 210

  L   T   A   D   K   S   S   T   T   A
TTGACCGCAG ACAAGTCCTC CACCACAGCC 240

  Y   M   Q   L   T   S   L   T   S   E
TACATGCAGC TGACCAGCCT GACATCTGAG 270

  D   S   S   V   Y   Y   C   A   R   E
GACTCTTCAG TCTATTACTG TGCAAGAGAG 300

  G   Y   D   G   F   D   S   W   G   Q
GGGTACGACG GGTTTGACTC CTGGGGCCAA 330

  G   T   T   L   T   V   S   S
GGCACCACTC TCACAGTCTC CTCA        360
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Fig. 2

```
  E   L   V   L   T   Q   S   P   A   I
GAGCTCGTGC TCACCCAGTC TCCAGCAATC  30
  M   S   A   S   P   G   E   K   V   T
ATGTCTGCAT CTCCAGGGGA GAAGGTCACC  60
  M   T   C   S   A   S   S   S   V   N
ATGACCTGCA GTGCCAGCTC AAGTGTAAAT  90
  Y   M   Y   W   Y   Q   Q   K   S   G
TACATGTACT GGTACCAGCA GAAGTCAGGC 120
  T   S   P   K   R   W   I   Y   D   T
ACCTCCCCCA AAAGATGGAT TTATGACACA 150
  S   K   L   A   S   G   V   P   A   R
TCCAAATTGG CTTCTGGAGT CCCTGCTCGC 180
  F   S   G   S   G   S   G   T   S   Y
TTCAGTGGCA GTGGGTCTGG GACCTCTTAC 210
  S   L   T   L   S   S   M   E   A   E
TCTCTCACAC TCAGCAGCAT GGAGGCTGAA 240
  D   A   A   T   Y   Y   C   Q   Q   W
GATGCCGCCA CTTATTACTG CCAGCAGTGG 270
  S   S   N   P   Y   T   F   G   G   G
AGTAGTAATC CGTACACGTT CGGAGGGGGG 300
  T   K   L   E   I   K
ACCAAGCTGG AGATAAAA              330
```

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Fig. 3

Eradication course of patient CXT0002